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A COMPARATIVE STUDY OF THE PEPTIZATION OF THE PROTEIN COMPLEX IN VARIOUS SEEDS AND GRAINS

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PHYSICO-CHEMICAL STUDIES ON PROTEINS

V. A Comparative Study of the Peptization of the Protein Complex in Various Seeds and Grains*

BY ERNEST V. STAKER AND ROSS AIKEN GORTNER

Historical

That certain proteins were soluble in saline solutions was first shown by Denis[1] in 1859. This observation was later confirmed by Hoppe-Seyler.[2] Denis observed that when sodium chloride extracts were made of both animal and vegetable tissue, certain proteinaceous substances were dissolved. While this discovery opened to chemists an entirely new means for isolating and purifying these proteins, its real significance was not appreciated until several years later.

In 1862 Ritthausen began his work on the vegetable proteins. For a number of years he devoted himself to the separation and purification of proteins and to their ultimate analysis. Ritthausen also became interested in that protein fraction which was extracted from seeds by weak alkaline solutions. As a result of these and other investigations, it became evident that the proteins in seeds existed in many diverse forms.

Weyl,[3] using the method of Denis, examined a large number of seeds and found that they all contained protein soluble in solutions of sodium chloride. Using his experiments as a basis, he divided the "globulin" thus extracted into two groups, the "vitellins," soluble in saturated solutions of sodium chloride, and the "myosins," insoluble in such solutions. Soon afterward, Vines[4] investigated the action of salt solutions on the aleurone grains in different seeds.

About this same time Weyl, who had been examining the data of others, made a severe criticism of Ritthausen's work. He maintained that the preparations which Ritthausen had obtained by extracting seeds with dilute alkali were altered products and that they did not represent the original constituents of the seed. Ritthausen soon afterward, however, showed that many of his preparations were largely or wholly soluble in saline solutions, and had for the most part retained unaltered their original solubility; also that the products obtained by direct extraction of seeds with neutral salt solutions agreed in many cases entirely with those he had previously described. Regardless of his assertion Ritthausen thought it best to apply the method of Denis to a number of seeds, especially those which he had previously studied, and he was able to show that most of the seed proteins were globulins. The experience of others has since substantiated this conclusion.

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It is difficult to understand why Ritthausen's preparations extracted from the seed by dilute alkali, and which he claimed to be of "highest purity" should later have been completely soluble in neutral saline solutions. If his samples had contained glutelins which are "insoluble" in salt solution but "soluble" in dilute alkali, they should not have become soluble in neutral salt solutions. One must conclude that either his proteins were almost wholly "globulins" and albumins and that no appreciable quantities of glutelins were present, or if originally present they had undergone partial hydrolytic cleavage into simpler fractions.

Thomas B. Osborne was the next investigator of importance in the field of the vegetable proteins. For more than twenty years he was primarily engaged in separating, classifying, and studying the protein constituents of seeds. His work included an extensive investigation not only of cereal grains but also of a large number of other kinds of seeds. Because of this fact the further historical review is divided into sections.

The Cereals

Barley.—As far as the authors are aware the salt- or water-soluble fractions of barley have been studied but little. Osborne[5] made the first preliminary survey. He observed that the seeds contained proteins which were soluble in water, in 10% sodium chloride, and in alcohol.

Osborne's conclusions from his investigation of the barley proteins were that "the barley contained 1.83% of nitrogen and if it be assumed that this all belonged to proteid matter with 17% of nitrogen, the flour would contain 10.75% proteids. The barley accordingly contained about 4.5% insoluble proteid, 4% of 'hordein' soluble in dilute alcohol, 0.3% albumin and 1.95% of globulin and proteose." If the albumin plus the globulin-proteose fraction is expressed in terms of the percentage of the total proteins, it is found that the total salt-soluble fraction amounts to 20.93% of the total protein. It will be noted also that the globulin fraction is 6.5 times that of the albumin fraction.

The only other paper since Osborne's studies on the salt-soluble proteins of barley, bearing directly on the subject, is that of Bishop[6] who recently reported some investigations on the composition and quantitative estimation of the barley proteins. His methods for the isolation of the globulin and albumin fractions are distinctly different from those employed by Osborne. Bishop extracted the seed meal with 5% potassium sulfate solution. The albumins in this extract were estimated by buffering the solution to pH 4.6 with an acetate-acetic acid buffer, and then coagulating the albumins at 82°C. The coagulum was filtered off and its nitrogen content determined. Total protein on the potassium sulfate extract was estimated by precipitation with trichloracetic acid and a subsequent nitrogen determination on the precipitate. The difference between the total protein nitrogen and the albumin nitrogen was assigned to "globulin" nitrogen.

Bishop found that approximately 34% of the total nitrogen in the seed was extracted when treated with 5% potassium sulfate solution. This is

approximately 13% greater than was found by Osborne, although Osborne made his extractions with 10% sodium chloride, using very different experimental conditions. Sodium chloride usually, however, extracts a greater percentage of the total protein than does potassium sulfate when equal concentrations of the salts are used. The non-protein nitrogen reported by Bishop (11.5-16.3% of the total nitrogen of the seed) is unusually high for sound grain, so much so as to cast serious doubt on the validity of his albumin and globulin values. Bishop found the globulin fraction to be much less than the albumin fraction, although Osborne reported that there was 6.5 times as much globulin as albumin in barley. We believe that this discrepancy lies not in the difference in chemical composition of the samples used by these investigators, but in the different methods which they employed.

Oats.—Norton[7] as early as 1848 recognized three proteins in the oat kernel, 0.5-2.17% of an albumin, 15.76-17.72% of "casein" (or avenine), and 1.33-2.47% of gluten.

Von Bibra[8] claimed the protein constituents to be an albumin (1.24%) precipitated by boiling the cold water extract of ground oats, casein (0.15-0.17%) the product separating from the hot alcoholic extract on cooling, plant gelatin (3.00-3.25%) the substance soluble both in hot and cold alcohol, and nitrogenous substances insoluble in water and alcohol 11.38-14.85%.

Osborne[9] was the first to extract oats with water and with 10% sodium chloride solutions. He observed that such extracts had a strong acid reaction and that upon neutralization a precipitate formed which he called acid albumin. After making several analyses of the water extract of oats he concluded that they contained (1) an acid albumin, (2) one or more globulins, and (3) a proteose which remained in solution after dialysis of the ammonium sulfate precipitate. The relative amounts of each of these stated either in terms of the weight of sample extracted or in percentage of the total nitrogen present, were not given.

In connection with his study of the oat kernel, Osborne's attention was called to the denaturizing effect of water. This was admirably demonstrated in the following experiment:

Osborne added 6 liters of water to 5 lbs. of "ground" oats. The mixture was allowed to stand over night when the supernatant liquid was decanted. Another 6 liters of water were added to the residual meal and the extraction repeated. The combined extracts were then saturated with ammonium sulfate, the precipitate separated from the remaining liquid, and suspended in water. This suspension was dialyzed in running water for 14 days. After filtering, the residue was extracted with a 10% sodium chloride solution, the solution again filtered and dialyzed. No precipitate of globulin formed, showing that all of the protein in the 10% sodium chloride solution was soluble in water. Osborne concluded that no globulin had been extracted from the substance which separated on dialysis of the aqueous extract, the globulin having been converted into an insoluble form. Osborne observed that such transformations were always the result of the use of water or salt solutions as contrasted with the use of alcohol, alkali, or heat: three agents known to

suspend or destroy enzyme action. He therefore suggested that the reason for the apparent anomaly referred to above was that the water had permitted enzyme action to proceed.

From numerous and varied experiments on the oat proteins, Osborne finally concluded that the primary constituents were: (1) an alcohol-soluble protein amounting to 1.25% of the total protein of the oat kernel, (2) a globulin amounting to 1.5%, and (3) an alkali-soluble protein which made up the remainder with the exception of extremely small amounts of proteose and acid-albumin.

Wheat.—Most of the very early work on wheat has little bearing on the present problem and will therefore not be reviewed. We again turn to Osborne[10] for much of our knowledge regarding the proteins of wheat.

Osborne has made a rather detailed study of the globulin and albumin fractions from the embryo and endosperm portions of the kernel. By extracting 3000 grams of germ meal with 9 liters of water and heating the filtrate to 65°C., he obtained 279 grams of albumin or 9.3% of the oil-free germ meal. In another experiment germ meal was extracted with water, the solution adjusted to neutrality by the addition of alkali, and dilute calcium chloride solution was added as long as a precipitate formed. The clear filtrate when heated to 65° gave rise to a coagulum, which, when dried, amounted to approximately 3.4% of the oil-free germ meal. It will be noted that this figure is only about one-third of the value given in the former experiment. The difference in yields was probably due to varying experimental conditions.

An estimation of the globulin fraction in the above experiment gave a value of 9.17% or 2.67 times as much as the albumin. In another determination where the quantity of globulin in the embryo was estimated by extracting 200 grams of the meal with 2000 cc. of 3% sodium chloride, a value of 5.05% of globulin was obtained. Still other studies indicated that 9.5% of the embryo was leucosin (albumin), 4.84% globulin, and 3.03% proteose.

Like differences are to be noted in the albumin fraction extracted from wheat flour. In one experiment flour was extracted with 10% sodium chloride, the solution filtered and the filtrate saturated with ammonium sulfate. The supernatant liquid was filtered and heated at 60-65° for 1 hour. The resulting coagulum upon washing and drying formed 0.064% of the original meal. In another experiment exactly like the preceding, except that dialysis was employed to precipitate the globulin and the albumin in the filtrate was coagulated at 90° instead of 60°, Osborne obtained a yield of 0.16% albumin. Osborne further determined the globulin: albumin ratio in the meal obtained from the entire kernel of winter and spring wheats. The meals were extracted with 10% sodium chloride solution, the extracts dialyzed to separate the globulins and the albumin in the residual solution coagulated by heat. Spring wheat was found to contain 0.624% globulin and 0.391% albumin, giving a globulin: albumin ratio of 1.60. Similar figures for winter wheat were 0.625% globulin and 0.359% albumin with a ratio of 1.74.

Considering the differences which might have resulted from using two different classes of wheat, the results check very closely. But it must be

emphasized that exactly the same method of procedure was used in each instance. This undoubtedly explains the very close agreement of the protein analyses of the two wheats.

Ladd[11] proposed a method for the determination of albumin and globulin in wheat and other cereals. Two hundred and fifty cc. of 1% sodium chloride solution was added to each 5 grams of flour. Total nitrogen was determined on a portion of the filtrate. To a like portion phosphotungstic acid was added, thus precipitating the true proteins and any organic nitrogen bases which may have been present in the extract. Nitrogen was then run on the filtrate, giving the "non-protein" nitrogen present in the extract. The albumin and globulin are then approximated by subtracting the non-protein nitrogen from the total nitrogen. This method assumes that only albumins and globulins are precipitated by phosphotungstic acid, whereas we know that other nitrogenous compounds including ammonia are precipitated.

Jones and Gersdorff[12] isolated and analyzed the globulin and albumin of bran. An extraction with water was first made at 1-2°C., using 20 cc. per gram of bran. This was continued until nothing more was removed. The combined extracts were dialyzed 12-14 days and the precipitated globulin removed. Further treatment of the filtrate with carbon dioxide eliminated the last traces of globulin. The filtrate was then boiled until coagulation of the albumin was complete. The residue from the water extraction was exhaustively extracted with 4% sodium chloride. The joint extracts were acidified with acetic acid and boiled until there was no further separation of globulin coagulum.

The percentage of the proteins isolated, expressed in terms of the total proteins in the bran, were: albumin, 16.64%; globulin, 13.62% with a globulin: albumin ratio of 0.83.

Bailey and Blish[13] made a study of the proteins extracted by diluted salt solutions from wheat flour. They estimate that only 48.3% of the protein nitrogen extracted by 1 per cent sodium chloride solution is referable to albumin and globulin, whereas 83.1% of the protein nitrogen extracted by 10% sodium chloride solution and 85.7% of the protein nitrogen extracted by 5% potassium sulfate solution are derived from albumin and globulin.

They believe that the extract of a patent flour when 1% sodium chloride is used as the solvent contains a large proportion of gliadin amounting to more than half of the total protein extracted. The extracts when 10% sodium chloride or 5% potassium sulfate solutions was used contained only 15% of the protein as gliadin. It was their conclusion therefore that 5% potassium sulfate solution should be used for the determination of the non-gluten proteins.

Hoffman and Gortner[14] have recently reported the results of some investigations in isolating the different proteins of wheat flour. The globulin fraction prepared by extracting flour with 5% potassium sulfate, and dialyzing the extract free of sulfate, was found to be practically insoluble in either 5% potassium sulfate or 10% sodium chloride solutions. It was soluble, however, in 0.2% sodium hydroxide. This product was designated as "globulin A." Since they thought that they might not have been dealing with a globulin in

this experiment, another preparation was attempted using 10% sodium chloride solution. This was called "globulin B." This product was found to be nearly all soluble in 10% sodium chloride solution. They point out the possibility that the dialysis of the final preparation may have been carried too far, thus causing a protein→protean transformation.

The filtrates after the globulins had been removed, were concentrated in vacuo and the albumins isolated by exactly the same procedure. The filtrate from "globulin A" yielded 21 grams of albumin and that from "globulin B" approximately 1 gram. Thus 5% potassium sulfate extraction gave a globulin: albumin ratio of 0.28, whereas 10% sodium chloride extraction gave a ratio of 3.0. The difference in these ratios must lie in the methods followed, since the same flour was used in both cases. In discussing these differences the authors add: "Certain experiences in making the separations reported in this paper, together with subsequent experience to be reported later, make us strongly doubt whether or not any apreciable amount of a true globulin exists in patent wheat flour. We are certain that if a globulin is present it is in extremely small amounts. We are equally certain that no appreciable amount of proteose was present in the sample of flour which we examined."

Corn.—To Gorham[15] must be given the credit for our first reference to the proteins of corn. From evaporating a clear alcoholic extract of corn, he obtained a yellow wax-like substance to which he gave the name "zein." He described zein as resembling gluten but differing from it in not containing any nitrogen, the nitrogen content being overlooked in his methods of analysis. Other early contributions were the observations of Ritthausen[16] concerning "maize fibrin," a substance soluble in alcohol, and the bare statement made by Th. Weyl[3] that "powdered seeds of maize yield to a 10% solution of sodium chloride a globulin-like substance which, after purification by repeated precipitation with water and resolution in 10% sodium chloride, coagulates at 75°."

Nothing further was done until Osborne[17,18] took up the question. He used as usual 10% sodium chloride for securing the saline extract. Osborne concluded that the maize kernel in addition to other proteins, contained three globulins, which together form 24.67% of the total nitrogen in the meal. It was his observation also that "through the long-continued action of strong solutions of salt, as ammonium sulfate, the myosin-like globulin, and the globulin with a still lower content of nitrogen, are changed into insoluble modifications, soluble, however, in 0.5% sodium carbonate solution from which they are precipitated by neutralization apparently as albuminates." This is evidence again of the denaturing action of water and salt solutions. Osborne also reached the conclusion that no true albumin existed in the maize kernel. This was a rather striking conclusion since he had found an albumin to be present in all the seeds previously examined.

Legumes

Beans (*Phaseolus* sp.).—Ritthausen[19] made water extracts of the white bean (*Phaseolus vulgaris*) as early as 1883. In the following year he made extractions with 2% sodium chloride. These were cleared by subsidence and

the residues, when dry, were reported as forming 13.2 and 11.45%, respectively, of the meal.

Osborne[20] extracted samples of kidney bean meal with 10% sodium chloride and concluded that it contained about 15% phaseolin, 2% phaselin, and 20.6% alkali-soluble protein. The first two were classified as globulins.

No further studies on beans were made until 1897 when Osborne[21] again turned his attention to the *Phaseolus* species. This time it was the small, red bean (*Phaseolus radiatus*) commonly grown in Japan. From 2 kg. of the ground seed treated with 8 liters of 10% sodium chloride solution he obtained 92.23 grams of globulin or 4.6% of the total weight of the meal. The percentage of nitrogen in the bean meal was not recorded.

Jones *et al*[22] investigated the proteins of the lima bean (*Phaseolus lunatus*). A 3% sodium chloride solution, at room temperature, extracted 72.32% of the total protein in the meal or 15.31% of the bean meal. They also noted that the soluble salts occurring naturally in the seeds were sufficient to dissolve 15.13% of protein when the meal was extracted with distilled water in the proportion of 2.5 cc. of water per gram of meal. This was practically as much as was extracted by the 3% sodium chloride solution. By fractional precipitation with ammonium sulfate they secured two globulins, α and β, which formed 2.74 and 1.58% of the total protein, respectively. An albumin, amounting to 1.75 per cent of the meal or 8.26% of the total protein, was obtained by coagulating by heat the residues of a distilled water extract of the meal after all of the globulins had been removed. This percentage of albumin is considerably higher than that obtained from the other beans which have been studied.

The proteins of the Mung bean (*Phaseolus aureus* Roxburgh) have been investigated by Johns and Waterman.[23] This bean they found to contain 21.74% protein of which about 87.5% was extracted by a 5% sodium chloride solution. Experiments with aqueous sodium chloride in different concentrations indicated a 5% solution as the most effective extractant; it dissolved 19.0% of protein from the finely ground seed. This extract yielded two globulins (designated α and β) by fractional precipitation with ammonium sulfate and subsequent purification. These fractions were equal to 0.35 and 5.75%, respectively, of the dry meal extracted. Only traces of an albumin were found in the meal.

Peas, Lentil, Horse Bean, Vetch.—In the early literature legumin was commonly referred to as a substance extracted from seeds by caustic alkalies, and more or less altered by the action of the solvent. After Ritthausen had repeated a number of his experiments, he concluded that his former preparations were a mixture of two proteins, one soluble in salt solutions after dissolving in dilute potassium hydroxide and precipitated with acid, and the other originally soluble in salt solution but rendered insoluble in that fluid by treatment with alkalies. This latter substance Ritthausen called "legumin."

Osborne and Campbell[24] pointed out that legumin forms the chief protein constituent of the pea, lentil, horse bean, and vetch. Vetch contained approximately 10% of this protein. These seeds also contain a globulin called

vicilin which is associated with legumin but difficult to separate from it. Osborne and Campbell found that when seeds containing legumin were extracted with water, the following percentages of that protein in terms of weight of the seed were dissolved: pea 4%, vetch 2.5%, lentil 10%, and horse bean 16%. Legumelin, an albumin, was found in all legumes studied with the exception of *Phaseolus vulgaris*. The pea contained 2%, vetch 1.5%, and horse bean 1.25%.

In another experiment,[25] where a large quantity of peas was extracted with 10% sodium chloride solution, a ratio of 1 part albumin to 3.42 parts of globulin was obtained. Unfortunately in many of his papers Osborne does not report the yields of his proteins. He was primarily interested in the nature of the proteins and in their analysis in contrast to the exact amount of the protein in the original biological material.

In their studies on the legume proteins Osborne and Harris[25] were able to show, "that from the pea, *Pisum sativum*, horse bean, *Vicia faba*, lentil, *Ervum lens*, and vetch, *Vicia sativa*, preparations of the globulin can be obtained which agree strictly in properties and composition with one another but are distinctly different from those obtained from seeds of the genus *Phaseolus* and other kinds of legumes."

In connection with his studies on the pea Osborne[26] made some 10% sodium chloride extractions of finely ground vetch. A globulin equivalent to 5.2% of the original meal was isolated. In another experiment, where water was used as the extracting agent the globulin equaled only 1.04% of the original meal.

Peanuts.—Until recently Ritthausen[27] was the only individual who had published any experimental work on the proteins of the peanut (*Arachis hypogea*). In 1880 this author extracted oil-free peanut meal with solutions of sodium chloride, potassium, calcium, and barium hydroxides. From the 10% sodium chloride extract he obtained 27% of globulin by saturating the diluted extract with carbon dioxide, or simply by diluting the extract with a large volume of water. Apparently the same globulin was obtained by acidifying the alkaline extracts with acetic or sulfuric acids. Ritthausen found no evidence of the presence of more than one globulin in the peanut.

The peanut meal used by Ritthausen had been dried over sulfuric acid in a desiccator and contained 10.18% of nitrogen or 56% of protein, using 5.5 as the nitrogen factor.

Lechnikov[28] found the total nitrogen in the peanut to be 9.1% of which 8.74% occurred as "albuminous substance," including albumin, gluten, and globulin.

Johns and Jones[29] extracted air-dried oil-free peanut meal containing 42% of protein (N × 5.5) with a 10% sodium chloride solution and found that about 32% of protein, based on the weight of the meal used, was dissolved at room temperature. When the temperature was raised to 40-50°C., the yield was not increased. Only a very small quantity of albumin was obtained.

Miscellaneous Seeds

Flax-seed.—Osborne[30] found that the proteins of ground flax seed which had been freed from oil by extraction with benzine or ether were largely soluble both in water and solutions of sodium chloride. He reported that flax seed contains a globulin precipitated by dialysis; a protein resembling both globulin and albumin precipitable by long-continued heating at 100°C., as well as by sodium chloride in the presence of acid; a proteose and peptone fraction, and a protein not extracted by sodium chloride but soluble in dilute alkali. Osborne states that "all attempts to determine the amounts of these substances failed because of change, while in solution, into non-proteid bodies. It was further found that the relative amount of the various proteids was very variable; it is almost certain that the more soluble forms were largely, if not wholly, derived from the globulin in consequence of alteration during extraction and separation." In one instance water extracted from 100 grams of meal, protein material equal to 10.5% of the weight of the meal. Forty grams of material, using a saturated sodium chloride solution as solvent, gave a yield of 3.7 grams of protein equal to 9.25% of the weight of the original meal.

Sunflower.—Prior to the work of Osborne, the only published observations on the proteins of the sunflower seed were made by Ritthausen[27] and by Vines.[31] By extracting with very dilute alkali, Ritthausen obtained from the finely ground oil-free meal 44.71% of protein. By treating with sodium chloride solution, diluting the extract with 5 volumes of water and passing carbon dioxide through the solution, the yield was 25.3%. He observed that a considerable part of the protein of the sunflower seed was insoluble in salt solutions but soluble in dilute alkali.

Osborne[32] extracted the oil-free sunflower meal with 10% sodium chloride solution and precipitated the protein by dilution, dialysis, or by saturating the solution with sodium chloride. He obtained 7.4% of a globulin, "edestin," and concluded that this was the principal protein of the seed.

Other seeds.—The salt- and water-soluble fractions of a number of other seeds have been studied. Johns and Chernoff[33] reported that distilled water at room temperature extracted 2.5% of protein from buckwheat flour, while 5 or 10% sodium chloride solutions extracted 4.5%. A 5% solution of magnesium sulfate extracted 3.8% of protein.

Jones *et al*[34] state that distilled water, 2% sodium chloride solution, and a 10% sodium chloride solution, extracted 15.0, 18.5, and 18.0% of protein, respectively, from the jack bean (*Canavalia ensiformis*). Johns and Finks[35] found that a globulin, obtained by extracting cocoanut meal with 10% sodium chloride solution, comprised 10% of the weight of the meal. Davies[36] observed that 25% of the total protein of the rape seed (*Brassica napus*) is extractable using a 10% sodium chloride solution. Other studies of this nature include those of Johns and Gersdorff[37] who studied the proteins of the cohune nut (*Attalea cohune*), that of Cajori[38] on proteins of the pecan nut, Jones[39] on proteins of the cantaloupe seed, and that by Takahashi[40] on the proteins of adzuki bean.

Proteins and the Lyotropic Series

The existence of the Hofmeister or lyotropic series of ions has been noted by Jaeger,[41] Frumkin,[42] Freundlich,[43] and others, under conditions where lyophilic or organic colloids were absent. Loeb[44] argued against such a series, at least in so far as protein behavior was concerned. However, Bancroft questioned the line of reasoning used by Loeb, and McBain stated: "The effects of the Hofmeister or lyotrope (Freundlich) series of ions are too striking to be ignored. They have been observed with too many types of colloids in connection with too many systems not even containing colloids to be attributed to systematic experimental error."

Gortner, Hoffman, and Sinclair[45] were the first to emphasize the effect of salt solutions of various concentrations on the differential solubility or peptization of proteins. Twelve samples of wheat flour were studied, including flours milled from the most important commercial classes of wheats. At almost constant hydrogen ion concentrations (pH 5.0 to 6.0) a marked lyotropic effect was observed. It is evident that the hydrogen ion concentration *per se* could not have been playing the major role, and that such peptization differences as they observed must be attributed to some other factor.

Considering the percentage of total protein peptized by the potassium halides, they found a very definite lyotropic effect of the order KF < KCl < KBr < KI. Considering those salts which had potassium as the cation, they found for N/2 concentrations a series F < SO_4 < Cl < tartrate < CrO_4 < Br; for N/1 concentrations F < SO_4 < Cl < tartrate < Br < I; and for 2 N concentrations F < Cl < Br. The same relative order held for all the salts studied.

These authors further point out that the results as obtained were not due to hydrolysis since there was no increase in free $-NH_2$ and $-COOH$ groups. The phenomenon must therefore have been purely a colloidal one involving differential and more or less specific peptization behavior.

In conclusion they remark that "there is a lyotropic series of ionic effects in an aqueous system of protein and salts, and that these effects are due to properties of the anion and the cation of the salt and are observable and measurable even at a constant hydrogen-ion concentration."

Pascoe, Gortner, and Sherwood[46] made extracts of various flour streams, using 0.5N solutions of KBr, KCl, KF, and $MgSO_4$. The grades of flour ranged from first break to bran and shorts duster. The data as presented substantiated in all respects the findings of Gortner, Hoffman, and Sinclair. Without a single reversal the ionic effect was F < Cl < Br.

Although the flours used differed not only among themselves in chemical composition and physical properties, but from normal wheat flours as well, the same distinct lyotropic effect as encountered by Gortner *et al* was manifest throughout.

Very few pure proteins have been studied in relation to their solubility in solutions of different salts. Osborne and Harris[47] were the first to make such a study. They investigated the action of salts on the globulin edestin ob-

tained from hemp-seed. This protein had been very carefully prepared in a pure crystalline state and was wholly insoluble in water.

Two grams of the air-dried preparation were suspended in a glass-stoppered bottle by adding a known quantity of a molar salt solution and sufficient water to make the final volume 20 cc. The mixture was then agitated at 20° for an unstated length of time and the undissolved globulin allowed to settle Ten cc. of the clear solution was drawn out with a pipette, its nitrogen content determined, and from this the amount of dissolved edestin calculated. By using successively larger quantities of the molar salt solution and correspondingly less water, a series of determinations was made. The amount of edestin, in every case, was in decided excess of that dissolved, so that presumably as nearly complete saturation of the solution as was possible, under the conditions of the experiment, was attained.

The chlorides of the monovalent bases sodium, potassium, and caesium were found to have very nearly the same solvent power. The divalent bases, barium, strontium, calcium, and magnesium, dissolved approximately twice as much edestin as the monovalent chlorides. The solubility in general was observed to be independent of the nature of the base, but proportional to the number of chlorine atoms. Lithium chloride proved to be the only exception, its solvent power being much less than that of the other monovalent chlorides.

The effect of sulfates on the solubility of the globulin was very similar to that of the corresponding chlorides of the divalent bases. A half-molar solution of sodium sulfate dissolved edestin freely while a full-molar solution dissolved scarcely any. With potassium sulfate the same effect was noticed, but owing to its limited solubility precipitation of the protein did not take place. The curve for lithium sulfate followed those of potassium and sodium sulfate until the latter began to fall. With lithium sulfate, however, the amount of dissolved edestin remained practically constant with increasing concentration of the salt solution, and no evidence of diminishing solubility appeared. Magnesium sulfate, though commonly considered to precipitate globulins, when added in sufficient amount, followed the same course as that taken by the chlorides of the divalent bases.

Solutions of sodium and potassium iodide each had the same solvent effect, which was much greater than that of solutions of the bromides of the same molecular concentration, and slightly greater than that of the chlorides of the divalent bases. Sodium and potassium bromide had nearly the same solvent power, which was considerably higher than that of corresponding solutions of the chlorides. The bromides of barium and calcium also had practically the same solvent power. In this case, however, it was only a little greater than that of the bromides of the monovalent bases, and was distinctly less than that of the chlorides of the divalent bases or of the sulfates. Lithium bromide, like lithium chloride, had less solvent power than the corresponding solutions of sodium and potassium bromide, but the same solvent power as solutions of sodium chloride of the same molecular concentration.

Osborne and Harris point out the similarity between the solubility of edestin in solutions of salts of strong bases with strong acids and that of the solu-

bility of certain insoluble inorganic salts in solutions of other salts due to the formation of complex salts in solution. They, therefore, thought it reasonable to assume that soluble addition products of the globulin and salt were also formed, a suggestion which had previously been made by Pauli.

In commenting further[48] on the problem of the solubility of proteins in salt solution, Osborne says: "Attention is called to these observations in the hope that someone may extend these investigations to other proteins with a view to developing better methods for extracting proteins from seeds than those now employed which depend almost entirely on the use of sodium chloride."

The above remarks were given little thought for a number of years. At least no well-planned experiments were undertaken in that direction. Just recently Sinclair,[49] realizing that much knowledge might be gained by following Osborne's suggestions, made a study of the solubility (peptizability) of pure gliadin in solutions of neutral salts.

Sinclair showed that normal solutions of various neutral salts peptized various amounts of gliadin. A normal KI solution peptized larger quantities of protein than did a normal solution of KBr, while the latter possessed a greater peptizing capacity than did a N KCl solution. When gliadin was treated successively with N KI solution, there remained some protein that was not capable of being peptized with N KI solution. However, if this non-peptizable protein was purified by repeated precipitations from alcohol in the same manner as the gliadin had been originally prepared the protein was then capable of being further peptized by N KI solution. The insoluble residue remaining after gliadin had been peptized the first time was repurified from salts by dialysis followed by solution in dilute alcohol and reprecipitation by absolute alcohol and ether. Following such repurification, it was indistinguishable from the original gliadin as judged by its repeptization by KI solution, analysis, etc.

Experimental

The Problem.—A review of the literature has disclosed the following points: (1) No systematic study has ever been made of the peptization of the proteins of seeds except those found in wheat flour and the protein, edestin (hemp seed); (2) There is a lack of quantitative data on the percentage of the total nitrogen extracted from seeds by water and by salt solutions; (3) The proportion of globulin to albumin in seeds has not been sufficiently studied; (4) Where such ratios have been computed using the data of other investigators, and considering the same kind of seed, a great variation has been noted. The investigation of the above problems, in so far as time would permit, formed the basis for the experiments reported in the following pages.

The Materials.—The seeds used were so chosen as to represent a number of the botanical families. In addition, they were selected so as to have them differ as widely as possible in their physical and chemical characteristics. They varied from 1.859 to 8.330 in percentage total nitrogen; from 1.850 to 70.72% in ether extract; and from 1.69 to 8.45% in ash content.

The plants from which the seed was obtained are listed below, grouped in accordance with the family represented.

Cereals

- Barley — *Hordeum vulgare*
- Oats — *Avena sativa*
- *Triticum* species
  - Alaska — *Triticum turgidum*
  - Einkorn — *Triticum monococcum*
  - Emmer — *Triticum dicoccum*
  - Club — *Triticum compactum*
  - Durum — *Triticum durum*
  - Marquis and Quality — *Triticum vulgare*
  - Polish — *Triticum polonicum*
  - Spelt — *Triticum spelta*

Leguminosae

- Common alfalfa — *Medicago sativa*
- Navy bean — *Phaseolus vulgaris*
- Alsike clover — *Trifolium hybridum*
- Andes Pea — *Pisum sativum*
- Peanut — *Arachis hypogea*
- Vetch — *Vicia angustifolia*

Cruciferae

- Mustard sp.
- Radish — *Raphanus sativus*
- Rape — *Brassica napus*

Miscellaneous

- Yellow dent corn — *Zea mays*
- White Ural millet — *Panicum miliaceum*
- Sweet sorghum — *Andropogon saccharatus*
- Teosinte — *Euchlaena Mexicana*

- Brazil nuts — *Bertholletia excelsa*
- Red Wing flax — *Linum usitatissimum*
- Hemp — *Cannabis sativa*
- Russian sunflower — *Helianthus annus*

Brazil nuts, radish, and vetch seeds were purchased on the open market. Those of hemp, teosinte, peanut, and vetch, were available at our own laboratories. The Alaska, White Polish, and Little Club varieties of the cereal grains were obtained through the courtesy of Dr. J. L. St. John, of the Washington State Agricultural Experiment Station. The remainder of the seeds or grains were furnished by the Department of Agronomy, University of Minnesota.

The seeds used had not been processed in any way and were in their natural state when secured. The seeds were bright in color and fully matured.

All samples before grinding were cleaned of dirt and other foreign material. As an extra precaution, and to make sure that the seed was all of one kind and type, the samples were "hand-picked."

In experiments involving the peptization of a plant material the necessity of standardizing the particle size is obvious. A survey of the literature showed that only one study had been made of the relation of particle size to the percentage of salt-soluble nitrogen. Bishop[6] observed that barley flour ground to pass a 1 mm. sieve yielded 25.6% of its nitrogen as "salt-soluble," when ground to pass a 1/2 mm. sieve this was increased to 31.2%, and when this material was reground in a "coffee mill" 36.2% of the nitrogen was extracted by the salt solution.

In our experiments it was thought best wherever possible to grind the samples sufficiently fine that they would all pass through a 100-mesh sieve. After grinding, the samples were thoroughly mixed so that uniform aliquots could be obtained. All samples were then stored at 0°C., until used.

A number of the seeds contained so much fat or oil as to make the fine grinding and sieving of the raw seed impracticable. Such seeds were first ground to a fineness commensurate with their oil content and then extracted in a large Soxhlet extractor with petroleum ether. After extraction and drying at room temperature, it was very easy to grind the material to the specified particle size.

The seed meals were analyzed for moisture, ash, and total protein. For the moisture determination 2-gram samples were dried at 100° to constant weight in the vacuum oven. Ash was determined on 5-gram samples, using the A.O.A.C. method. Total nitrogen was determined on 1-gram samples by the usual Kjeldahl-Gunning procedure.

The Methods.—(a) *Peptization of the Seed Proteins.*—The method used was essentially the same as that followed by Gortner *et al.*[45] Six grams of seed meal were weighed into a heavy-walled 100 cc. centrifuge tube and 50 cc. of the salt solution was added. The tube was then shaken in a mechanical shaker for exactly 30 minutes. Following the shaking, the meal was packed tightly in the bottom of the tube by whirling in a centrifuge, centrifuging being continued until the supernatant liquid was clear. This clear liquid was then carefully decanted into a Kjeldahl flask. After loosening the residue in the tube by means of a stirring rod, a fresh 50 cc. of salt solution was added, and a second extraction made in exactly the same manner as the first. The

contents of the tube were again centrifuged and the clear supernatant liquid decanted into the same Kjeldahl flask. A third extraction was made in a similar manner. The nitrogen dissolved by the three successive salt extractions was then determined on the combined extracts by the usual Kjeldahl-Gunning method. Duplicate extractions were made in all instances. In case the usual limit of error for a Kjeldahl determination was exceeded, the extractions were repeated on fresh samples of meal until satisfactory analytical agreement was obtained. Half-gram samples were employed for the legumes and 1-gram samples for corn, in that section of the work where a comparison was made between the percentage of the total nitrogen peptized in unextracted meal and that in ether-extracted meal.

(b) *Extraction and Separation of the Albumin and Globulin Fractions.*— Ten-gram samples of the various meals were weighed into 250 cc. wide-mouth bottles and 200 cc. of distilled water was added. A single extraction of the meal was then made by shaking the bottle in the mechanical shaker for 2 hours and centrifuging as usual. Total nitrogen was then determined in duplicate on 25 cc. aliquots of the clear supernatant liquid. A 100 cc. portion of the supernatant liquid was placed in a cellophane dialyzing tube, and dialyzed against repeated changes of distilled water for 60 hours (room temperature) using toluene as a preservative. Upon completion of the dialysis, the contents and washing of the casing were poured into a beaker and the resulting mixture filtered. The filtrate and washings from the precipitate were caught in a 250 cc. volumetric flask, the solution made up to volume and total nitrogen determined in duplicate on aliquots.

Only in the case of flax was it necessary to use the centrifuge instead of a filter to separate the globulin from the albumin liquid. The water extract of the flax meal contained so much gummy material as to make filtering impossible.

The meal residue remaining from the original water extract was treated twice more with 200 cc. portions of distilled water. As usual the bottles were shaken two hours for each extraction and then centrifuged. These extracts were discarded. It was assumed that such a procedure would remove the remainder of the albumin contained in the sample and that whatever protein might be dissolved by further treatment with salt solutions would be globulins. Accordingly, the meal residues remaining after three water extractions were subsequently treated with M/2 KCl solution. In order to make the results strictly comparable to those where salt extractions had been made on 6-gram samples, 85 cc. of the salt solution was added to each sample. Three separate extractions of 30 minutes each were made and the extracts decanted into the same Kjeldahl flask and a total nitrogen determination was made.

The residues from the KCl extraction were re-extracted using 0.5 M KBr solution. Here again three extractions were made, the residues being treated each time with 85 cc. of the salt solution. These data form a part of that recorded in Table VI.

We have already noted in the historical review that different globulin : albumin ratios are frequently obtained where two different methods are employed

TABLE I

The Percentage of Total Nitrogen peptized from Seed Meals by Various M/2 Salt Solutions
Six-gram Samples used

Classification	Seed used	Moisture	Ash	Total Nitrogen	Total Nitrogen Peptized by***				
					KF	K_2SO_4	KCl	KBr	KI
		%	%	%	%	%	%	%	%
Cereals	Barley	7.80	3.13	2.634	24.50	27.77	28.51	31.84	33.33
	Oats	6.58	4.25	1.859	25.35	30.42	34.78	46.49	55.30
	Tr. Species (Av.)	9.67	2.09	2.76	24.86	26.24	30.98	40.16	55.01
	T. Vulgare (Av.)	10.04	1.90	2.288	29.79	31.61	36.88	46.14	57.02
Legumes	Common alfalfa*	8.08	3.81	6.044	49.68	65.05	59.08	66.74	73.69
	Navy beans*	12.66	3.96	3.768	82.41	83.26	85.45	84.29	90.61
	Alsike clover*	8.03	3.94	6.037	53.83	64.13	56.34	63.72	76.22
	Andes field pea*	10.46	3.51	4.215	85.23	83.93	84.05	88.33	95.18
	Peanut	8.89	4.37	8.242	65.99	89.19	86.09	89.51	93.19
	Vetch	8.72	2.84	5.266	70.66	73.08	71.49	74.48	77.96
Cruciferae	Mustard Sp.**	6.98	4.75	4.889	57.56	59.41	60.05	59.66	58.19
	Radish	7.31	4.31	5.774	72.36	73.11	74.74	76.57	77.43
	Rape	8.19	4.47	4.322	67.71	70.78	72.73	74.59	74.61
Miscellaneous	Yellow dent corn	8.48	1.81	1.98	19.39	19.72	19.72	21.38	22.01
	White ural millet	8.35	4.02	2.187	9.79	11.87	11.91	12.55	12.54
	Sweet sorghum	9.18	2.29	1.978	8.24	7.91	7.93	7.96	8.03
	Teosinte	6.01	2.69	1.880	6.22	6.42	6.39	7.05	7.69
	Brazil nut	8.81	5.96	5.323	81.58	82.46	82.13	82.97	83.19
	Red Wing flax	8.23	4.86	5.050	53.53	65.26	57.64	63.51	60.94
	Hemp	8.02	8.37	5.716	24.65	46.39	30.32	57.42	70.58
	Russian sunflower	7.07	3.24	2.849	41.18	49.44	56.00	58.67	64.90

* 0.5 grams samples.

** The values recorded for mustard are probably not representative of any single species, since the seed was undoubtedly a mixture of various mustard species.

*** The peanut, mustard, radish, rape, corn, flax, sunflower, and Brazil nut meals were extracted with petroleum ether prior to peptization of the meal by salt solutions.

TABLE II

Comparison of the Percentages of the Total Nitrogen in Extracts of Various Seed Meals
Ten-Gram Samples Used*

Classification	Seed used	Nitrogen Water Extract A	Nitrogen remaining in Solution following Dialysis of Water Extract B	Nitrogen peptized by M/2 KCl following Water Extrac-tions C	Globulin extracted by Water A−B=D	Total Globulin C+D=E	Total Albumin + Globulin B+E=F	Peptized by KCl (no water extraction) G	Globulin denatured G−F=H	Globulin / Albumin D/B=I
		%	%	%	%	%	%	%	%	
Cereals	Barley	15.12	3.74	7.05	11.38	18.43	22.17	28.51	6.33	3.04
	Oats	16.77	1.73	6.62	15.04	21.66	23.39	34.78	11.38	8.69
				9.12**		24.16**	25.89**	46.48**	20.59**	
	Tr. species (Av.)	20.01	4.41	9.40	15.60	25.00	29.41	30.98	1.57	3.53
Leguminosae	Alfalfa	41.80	3.29	8.52	38.51	47.03	50.32	59.08***	8.76	11.70
	Navy beans	73.15	14.11	9.29	59.04	68.33	82.44	85.45	3.01	4.18
	Alsike clover	25.16	3.42	14.05	21.74	35.79	39.21	56.34***	17.13	6.36
	Andes field pea	78.87	10.86	10.09	68.01	78.10	88.96	84.05	−4.91	6.26
	Wild vetch	25.48	4.02	35.62	21.46	57.08	61.10	71.49	10.38	5.34
	Peanut	66.69	5.36	4.57	61.33	65.90	71.26	86.09	14.83	11.44
Cruciferae	Radish	49.67	8.46	21.58	41.21	62.79	71.25	74.74	3.49	4.87
	Rape	47.80	12.37	19.55	35.43	54.98	67.35	72.73	5.38	2.86
Miscellaneous	Yellow dent corn	12.59	3.34	7.17	9.25	16.42	19.76	19.72	−0.04	2.77
	White Ural millet	5.94	1.43	3.70	4.51	8.21	9.64	11.91	2.27	3.15
	Sorghum	8.29	0.83	0.42	7.46	7.88	8.71	7.93	−0.78	8.99
	Teosinte	4.98	0.83	0.92	4.15	5.07	5.90	6.39	0.49	5.00
	Brazil nut	35.33	8.40	37.94	26.93	64.87	73.27	82.13	8.86	3.21
	Red Wing flax	64.07	15.64	10.38	48.43	58.81	74.45	57.64	−16.81	3.10
	Hemp	13.63	1.31	9.51	12.32	21.83	23.14	30.32	7.18	9.40
	Russian sunflower	37.65	12.74	10.21	24.91	35.12	47.86	56.00	8.14	1.95

* The technic of extraction employed in this table differs from that employed in the case of Table I, in that in the present table a 10-gram sample was given a single extraction with 200 cc. of water (or salt solution) during a two-hour period.

** KBr extraction.

*** 0.5 gram samples used.

TABLE III

The Percentage of the Total Nitrogen Peptized from Various Wheat Meals by Distilled Water and by M/2 Salt Solutions

Wheat Variety	Moisture	Ash	Total Nitrogen	Total Nitrogen Peptized by					
				H_2O	KF	K_2SO_4	KCl	KBr	KI
	%	%	%	%	%	%	%	%	%
Alaska	9.73	1.96	2.729	19.58	21.86	23.74	28.29	38.07	54.80
Einkorn	9.37	2.22	2.891	17.89	22.39	23.27	27.67	36.28	56.42
Emmer	9.32	2.25	2.477	21.28	21.08	22.65	28.33	38.73	52.99
Little Club	10.57	2.25	2.99	19.72	25.97	27.10	30.61	38.76	53.55
Marquis	10.50	1.69	2.464	23.16	26.84	28.63	33.24	42.57	54.40
Mindum	10.47	2.25	3.017	21.58	24.93	26.01	30.91	41.18	58.09
Polish	8.34	1.81	3.64	18.34	21.97	23.20	27.39	36.42	55.53
Quality	9.58	2.11	2.112	26.72	32.74	34.59	40.53	49.72	59.65
Spelt	9.18	2.28	2.509	23.26	25.94	26.93	31.83	39.75	49.63
Average	9.67	2.09	2.76	21.28	24.86	26.24	30.98	40.16	55.01

in isolating the albumin and globulin fractions of a seed. To ascertain whether or not the same ratio of globulin to albumin would be found using a salt solution as was found in the distilled water experiments, the extractions were repeated on 10 grams of the seed meal of corn, millet, sorghum, and teosinte, extracting with 200 cc. of 0.5 M KCl solution. The method followed in the separation of the albumin and globulin fractions was exactly the same as that described above. Dialysis was continued until there was no further test for chloride in the dialysate. The data are shown in Table VII.

All data contained in the tables were computed on the moisture-free basis. Wherever extractions were made, the nitrogen thus dissolved was expressed in terms of percentage of the total nitrogen of the meal. In case the oil or fat had been removed previous to peptization this percentage was again added to the total dry matter and the results computed on that basis.

The Data.—The data are contained in Tables I to XI and in Figs. 1 and 2. The graphs, as diagrammed, are not mathematically correct, since there is only one variant. However, it is felt that for the purpose intended, they represent the data much better than would a graph of the "block" type.

TABLE IV

Relative Differences in the Percentage of the Total Nitrogen of Wheat Sorts which is peptized by Various Salts*

Wheat Variety	Difference between the Percentage of the Total Nitrogen Peptized by				
	K_2SO_4 and KF	KCl and K_2SO_4	KBr and KCl	KI and KBr	KI and KF
Alaska	1.88	4.55	9.78	16.73	32.94
Einkorn	0.88	4.40	8.61	20.14	34.03
Emmer	1.57	5.68	10.40	14.26	31.91
Little Club	1.13	3.51	8.15	14.79	27.58
Marquis	1.79	4.61	9.33	11.83	27.56
Mindum	1.08	4.90	10.27	16.91	33.16
Polish	1.23	4.19	9.03	19.11	33.56
Quality	1.85	5.94	9.19	9.93	26.91
Spelt	0.99	4.90	7.92	9.88	23.69
Average	1.38	4.74	9.18	14.85	30.15

* The first salt named in the column peptized the largest amount of nitrogen.

TABLE V

A Comparison of the Various Nitrogen Fractions expressed as per cent of the Total Nitrogen and the Albumin:Globulin Ratio obtained from the Extracts of Wheat Meals

Wheat Variety	Total Nitrogen peptized by: Water* (Albumins + Some Globulin in Soln.)	Water Following Dialysis (Albumins in Soln.)	M/2 KCl Following Water Extractions (Globulin)	Globulins extracted by Water	Total Globulin	Total Albumin + Globulin	Extraction originally by M/2 KCl (No water Extraction)	Globulin denatured by dialysis and which then appears in the Glutelin	Globulin / Albumin
	A	B	C	A−B=D	C+D=E	B+E=F	G	H	D/B
	%	%	%	%	%	%	%	%	
Alaska	19.18	4.70	8.71	14.48	23.19	27.89	28.29	0.40	3.08
Einkorn	16.67	2.77	10.91	13.90	24.81	27.58	27.67	0.09	5.02
Emmer	20.39	5.00	8.29	15.39	23.68	28.68	28.33	−0.35	3.08
Little Club	18.94	4.91	10.05	14.93	24.98	28.99	30.61	1.62	3.72
Marquis	21.21	7.04	8.08	14.17	22.25	29.29	33.24	3.95	2.01
Mindum	19.34	5.94	8.57	13.40	21.97	27.91	30.91	3.00	2.26
Polish	17.47	3.74	8.54	13.73	22.27	26.01	27.39	1.38	3.67
Quality	24.94	3.78	13.01	21.16	34.17	37.95	40.53	2.58	5.60
Spelt	21.92	2.69	8.42	19.23	27.65	30.34	31.83	1.49	7.15
Average	20.01	4.41	9.40	15.60	25.00	29.41	30.98	1.57	3.53

*The values in this column differ somewhat from values in the similar column of Table III, because a different technic was employed. In Table III the data represent the extractions of six-gram samples with three successive treatments of 50 cc. of water, each treatment with water being for 30 minutes. In the present table a *single* extraction with 200 cc. of water during a two-hour period was employed.

TABLE VI

The Percentage of the Total Nitrogen peptized from the Same Charge of Seed Meal using Successive Extractions with Different Reagents

Seed used	Total Nitrogen peptized by				Ratios		
	Distilled Water*	M/2 KCl following Water Extract†	M/2 KBr following Extraction by M/2 KCl‡	Total extracted	A/B	B/C	A/C
	A	B	C	A + B + C			
	%	%	%	%			
Barley	15.12	7.05	3.38	25.55	2.14	2.09	4.47
Alsike clover	25.16	14.05	2.79	42.00	1.79	5.04	9.02
Red Wing flax	64.07	10.38	4.50	78.95	6.17	2.31	14.24
Hemp	13.63	9.51	3.86	27.00	1.43	2.46	3.53
Oats (sample 1)	16.77	6.62	5.25	28.64	2.53	1.26	3.19
Oats (sample 2)	16.81	9.12**	2.58***	28.51	1.84	3.53	6.52
Wild vetch	25.48	35.62	6.88	67.98	0.71	5.18	3.70

* Ten-gram samples submitted to single extractions, using 200 cc. of water for a two-hour extraction period.

** KBr

*** KCl

† Residue from water extraction peptized with three successive portions of 85 cc. 0.5 M KCl, each treatment being for a period of 30 minutes.

‡ Residue from KCl extraction peptized with three successive portions of 85 cc. 0.5 M KBr, each treatment being for a period of 30 minutes.

TABLE VII

Comparison between the Globulin/Albumin Ratios when Extractions were made with 0.5 M KCl and Distilled Water, respectively

Seed Used	Fraction of Total Nitrogen peptized by M/2 KCl*	Nitrogen Remaining in Solution Following Dialysis of KCl Extract	Globulins	Ratios: Globulin / Albumin from KCl Extracts	Ratios: Globulin / Albumin from Water Extracts (Table II)
	A	B	A-B		
	%	%	%		
Yellow dent corn	20.10	4.17	15.93	3.82	2.77
White Ural millet	11.57	1.96	9.61	4.90	3.15
Sweet sorghum	7.79	1.24	6.55	5.28	8.99
Teosinte	7.13	2.08	5.05	2.43	5.00

* The values in Column A differ from those in Table I and Column G, Table II, because of a somewhat different technic employed in the extraction. In Tables I and II, the data represent the extractions of six-gram samples with three successive treatments of 50 cc. of M/2 KCl, each treatment with M/2 KCl being for 30 minutes. In the present table a single extraction of 10 grams of the meal with 200 cc. of M/2 KCl during a two-hour period was employed.

TABLE VIII

Comparison of the Ratio of the Total Nitrogen peptized by M/2 KCl and M/2 KI, respectively, and the Percentage of the Total Protein appearing in the Globulin Fraction

Classification	Seed used	Ratio of KCl/KI	Total Globulin %
Cereals	Barley	0.855	18.43
	Oats	0.628	21.66
	Wheat (av.)	0.563	25.00
Leguminosae	Alfalfa	0.801	47.03
	Navy beans	0.943	68.33
	Alsike clover	0.739	35.79
	Andes pea	0.883	78.10
	Hairy vetch	0.917	57.08
	Peanut	0.923	65.90
Cruciferae	Radish	0.965	62.79
	Rape	0.974	54.98
Miscellaneous	Corn	0.895	16.42
	Millet	0.949	8.21
	Sorghum	0.987	7.88
	Teosinte	0.830	5.07
	Brazil nut	0.987	64.87
	Flax	0.945	58.81
	Hemp	0.429	21.83
	Sunflower	0.862	35.12

TABLE X

The Percentages of the Total Nitrogen peptized by M/2 Salt Solutions from Whole Seed Meals and from Seed Meals which had been exhaustively extracted with Ether

Seed Meal Used	Nature of Sample	Ether Extract removed	Percentage of Total Nitrogen peptized by:				
			KF	K_2SO_4	KCl	KBr	KI
		%	%	%	%	%	%
Navy beans	Whole meal	——	82.41	83.26	85.45	84.29	90.61
Navy beans	Extracted meal	1.85	86.71	88.97	87.94	88.48	93.10
Navy beans	Difference*	——	+4.30	+5.71	+2.49	+3.79	+2.49
Alsike clover	Whole meal	——	53.82	64.12	56.34	63.72	76.22
Alsike clover	Extracted meal	9.48	55.09	70.04	63.89	70.83	76.61
Alsike clover	Difference	——	+1.27	+5.92	+7.55	+7.11	+0.39
Andes field pea	Whole meal	——	85.22	83.94	84.05	88.34	95.18
Andes field pea	Extracted meal	1.45	85.59	91.22	89.33	90.15	95.13
Andes field pea	Difference	——	+0.37	+7.28	+5.28	+1.81	−0.05
Yellow dent corn	Whole meal**	——	15.84	16.69	16.92	18.48	19.96
Yellow dent corn	Extracted meal	4.30	19.16	19.29	19.24	20.13	21.47
Yellow dent corn	Difference	——	+3.32	+3.40	+2.32	+1.65	+1.51
Alfalfa	Whole meal	——	49.68	65.05	59.08	66.74	73.69
Alfalfa	Extracted meal	11.72	49.95	65.48	56.57	61.37	69.34
Alfalfa	Difference	——	+0.27	+0.43	−2.51	−4.37	−4.35
Hemp	Whole Meal	——	24.65	46.39	30.32	57.42	70.58
Hemp	Extracted meal***	0.61	26.17	64.44	41.54	63.69	71.55
Hemp	Difference	——	+1.52	+18.05	+11.22	+6.27	+0.97

* A + sign indicates that a greater peptization of the proteins occurs in the ether-extracted meal.

** These values for corn differ from those reported in Table I probably because of the fact that the meal used in the extractions reported in Table I had been extracted with petroleum ether.

*** The "whole meal" was from a larger lot originally prepared in our laboratories for other purposes. It had been washed in gasoline to remove the greater proportion of the oil so as to prevent rancidity.

TABLE IX

Comparison of the Ratio of the Total Nitrogen peptized by M/2 KCl and M/2 KI, respectively, and the Percentage of the Total Protein appearing in the "Globulin" Fraction

Wheat	Ratio of KCl/KI	Total Globulin %
Alaska	0.516	23.19
Einkorn	0.490	24.81
Emmer	0.534	23.68
Little Club	0.571	24.98
Marquis	0.611	22.25
Mindum	0.532	21.97
Polish	0.493	22.27
Quality	0.679	34.17
Spelt	0.641	27.65

Discussion

Peptization of the Proteins of Barley and Oats.—The first part of Table I shows the percentage of the total nitrogen peptized for the grains, barley and oats. The same data are illustrated graphically in Fig. 2. A very definite lyotropic effect of the order $KF < K_2SO_4 < KCl < KBr < KI$ is noted, the same order being followed by both grains. In every case, however, a greater percentage of the total nitrogen of oats is peptized than is the case with barley. It will be further noted that a M/2 solution of KF peptizes almost the same percentage of the total nitrogen of both cereals. However, as we proceed from the salt which peptizes the smallest amount (KF) to that peptizing the largest (KI), the difference between the same salts becomes increasingly greater. Thus the difference between the fluorides is 0.85%; between the sulfates 2.65%; the chlorides 6.27%; the bromides 14.65%; and the iodides 21.97%. The peptization range of barley is from 24.50% (KF) to 33.33% (KI), a difference of only 8.83%. When the same comparison is made with oats it is found to be from 25.35% (KF) to 55.30% (KI), a difference of almost 30%.

A glance at the graph illustrating these relationships shows that almost a straight line can be drawn for oats when the halogen salts are considered. Such a line cannot be drawn for barley, as all the points are best connected with a curved line. It will also be observed that the behavior of the proteins of oats toward salt solutions resembles that of the proteins of wheat. In fact if the curve for oats is compared with that for the average of the *Tr. vulgare* group of wheats, a very similar relationship is seen.

The question at once arises as to why the proteins of these cereals react so differently. It is evident that since the proteins from these grains were subjected to exactly the same kind of treatment, the differences found must be attributed largely to the properties of the proteins themselves. Other factors could enter which might influence the total amounts peptized by the

various salts, but it is difficult to see how these would account for the difference in behavior when one cereal is compared with another. Reference is made here especially to the fat content of the seeds, a point which will be considered later.

If the chromosome numbers of barley, oats, and wheat are compared in relation to the peptization curves for these cereals, another relationship may

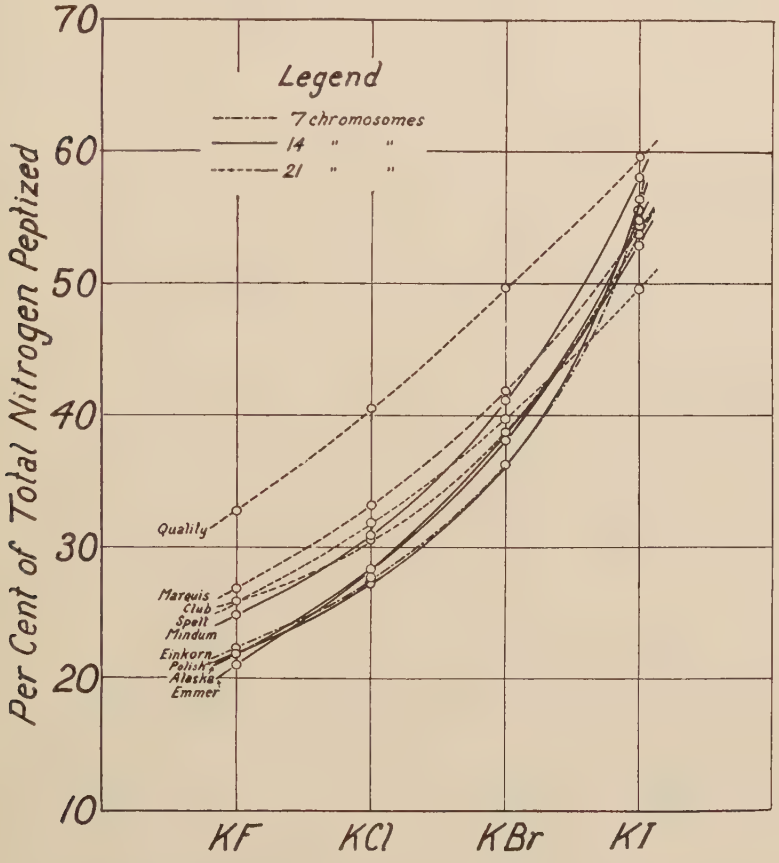

FIG. 1

Peptization of the Proteins of Various Triticum Species by 0.5 molar salt solutions.

be seen. The cultivated varieties of barley have 7 chromosome pairs, those of oats 21 and the *vulgare* species of wheats 21. The curve (Fig. 2) showing the average peptization for all the types or varieties of wheats is observed to be about midway between that for the *vulgare* class and the curve for barley. The only exception to this is the upper part of the wheat-average curve. Reference to Fig. 1 will show that it is those wheats which have the lower number of chromosomes, either 7 or 14, that bend the wheat-average curve toward the curve for barley. One might conclude then, that as far as the data presented here are concerned, ease of peptization of the wheat proteins seems to be associated with a correspondingly larger number of chromosomes.

The variation in the percentage of total nitrogen occurring in barley, oats, and wheat does not, apparently, account for the differences described above nor for the relative amounts of total nitrogen peptized. Oats contained the smallest amount of total nitrogen (1.859%) yet, with the exception of the *T. vulgare* group of wheats, a larger percentage of it was peptized than was true of either barley or the average for all of the *Triticum* species.

Peptization of the Proteins of Wheat.—In Table III and Fig. 1 are recorded the data obtained by peptizing the proteins of a number of wheats, most of which represent a distinct type. The fifth column of the table shows the percentage of the total nitrogen peptized by distilled water and was included for comparison with the amounts peptized by the various salts. Here again we have a marked lyotropic effect of the order $F < SO_4 < Cl < Br < I$. There is not a single reversal of this arrangement, although 10 different varieties of wheats were studied and 5 different salts employed.

If the peptization with one salt is compared with the peptization of another, proceeding from left to right across the table, it will be found that in a general way these differences are relative differences. These values have been recorded in Table IV. The differences as tabulated were obtained by subtracting the salt which peptized the least amount of nitrogen from the next one above it which peptized a larger amount. In Column 6 appear the figures found by subtracting the data for KF from that of KI. In this manner the differences are all positive. The last row of figures at the bottom of the table record the average differences between the various groups of salts. It is thus seen that there is less average difference between the peptization by K_2SO_4 and KF, than between KCl and K_2SO_4. The greatest variance is found, as one would expect, between KI and KF.

The data support the conclusion that considering all the wheats as a whole, their peptization behavior is very similar. Fig. 1 shows that while this statement is true generally, there are some differences. It will be seen that the grains divide themselves into three specific groups. The lower group includes White Polish, Emmer, Einkorn, and Alaska; the middle group comprises the varieties Spelt, Club, Durum, and Marquis, and the upper contains only one variety, Quality. This division, with the exception of Quality, is only true, however, with reference to the behavior of these grains toward the salts, KF and KCl. When the KBr level has been reached these differences have largely disappeared and nothing of this nature is observed when the peptization with KI is considered. In fact most of the lines have crossed one another.

It was thought that the division of the wheats into three groups might correspond to the classification of those grains which is sometimes made on the basis of their chromosome number. Sax[50] in confirmation of the studies of Sakamura[51] and Kihara[52] list the haploid numbers of the wheat group as follows:

Triticum monococcum	Einkorn group	7 chromosomes
T. dicoccum		
T. durum		
T. turgidum	Emmer group	14 chromosomes
T. polonicum		
T. spelta		
T. vulgare	Spelt group	21 chromosomes
T. compactum		

Reference to the graph will show that the grains are not thus divided on the basis of protein peptizability. It is to be noted, however, that three wheats of the Emmer group, *i.e.*, *T. dicoccum*, *T. turgidum*, and *T. polonicum* are found in the lower division. Einkorn is also found there, whereas if a strict subdivision were maintained on the basis of the chromosome number, it should form a group by itself. *T. durum*, theoretically, should belong to the Emmer group. However, it shows the peptization behavior of the Spelt wheats.

With the exception of *T. durum*, the Spelt group corresponds to the classification made on the basis of chromosome number. It must be borne in mind that Quality is a soft wheat. Had a number of wheats of this nature been included in the study, the gap between Quality and the Spelt wheats might not have been so apparent.

The average results for the entire group or for the *T. vulgare* varieties are higher than those reported by Gortner, Hoffman, and Sinclair.[45] This might have been expected, since our results were based upon the peptization of the proteins from the kernel, while theirs included only the proteins from wheat flour. Our results are likewise higher than those reported by Pascoe, Gortner, and Sherwood[46] who extracted various flour streams with 0.5 M salt solutions. It is interesting to note, however, that as the values given by them for the lower grades of flour are approached there is in general an increase in the percentage of the total nitrogen peptized. However, these values are much lower than the average we have obtained from whole wheat meal, the same salt being considered.

Peptization of the Proteins of Legumes.—The results obtained from peptizing the seed meals of various legumes are shown in Table I and Fig. 2.

Again we have evidence of a lyotropic series, the order of the anions being as follows: alfalfa, peanuts, and vetch, $F < Cl < SO_4 < Br < I$; beans, $F < SO_4 < Cl < Br < I$; clover, $F < Cl < Br < SO_4 < I$; peas, $SO_4 < Cl < F < Br < I$. This order is not as consistent as was found for the cereal grains. The discrepancy, however, is largely due to the potassium sulfate, the position of which varies from seed to seed. If this salt is omitted, the halogen salts assume the same order as before: $KF < KCl < KBr < KI$. There is only one exception to this arrangement and that is in the case of the pea, where the order of the fluoride and chloride anions is reversed. Table I shows that KF

peptized 85.23% of total nitrogen, while KCl peptized 84.05%. It was thought that the value for KF might have been too high, but four different subsequent determinations of this value gave results which checked within experimental error of that percentage.

A much larger percentage of the total nitrogen of the legumes is peptized than is the case with the cereal grains. In fact the lowest value for KF in the case of the legumes is almost as high as the KI value for any cereal. This may be partially accounted for by the large amount of total nitrogen found in the legumes as contrasted with the smaller amount contained in the cereals.

The peptization range, together with the resulting difference between the peptization by the salt having the lowest value and that having the highest, is shown in Table XI. In the case of the pea if the value for KF instead of K_2SO_4 is taken as the minimal per cent, the difference becomes 9.96% instead of 11.24%. In so far as these differences are concerned the legumes divide themselves into two groups; beans, peas, and vetch form one, while alfalfa, clover, and peanuts form the other group.

TABLE XI

Showing the Maximum, Minimum, and Difference in the Percentage of the Total Nitrogen of the Legume Seed Meal peptized by Salt Solutions

Seed used	Minimum	Maximum	Difference
	%	%	%
Alfalfa	49.68	73.69	24.01
Beans	82.41	90.61	8.20
Clover	53.82	76.22	22.40
Peanuts	65.98	93.19	27.21
Peas	83.94	95.18	11.24
Vetch	70.66	77.96	7.30

The relationships between the various legumes are illustrated more clearly in Fig. 2. The curves for the pea and bean are quite similar and occupy about the same position in the graph. Alfalfa and clover are likewise similarly placed, but the curve for alfalfa is practically the reverse of that for clover. Vetch occupies a midway position. So far as the shape of the curves are concerned, it will be noted that the curves for pea, bean, vetch, and clover bear a resemblance to one another. Alfalfa and peanut have curves which are practically the reverse of those just mentioned but which, when compared with one another, are found to be of the same general character.

Peptization of the Proteins of Cruciferae.—The *Cruciferae* studied included the mustard, radish, and rape seeds. The data appear in Table I and in Fig. 2.

As in the legumes these seeds are characterized by the ready peptization of a high percentage of the total nitrogen. The lyotropic order of anions is mustard, $F < I < SO_4 < Br < Cl$; radish and rape, $F < SO_4 < Cl < Br < I$.

Mustard shows a marked difference in lyotropic behavior when compared with the radish and rape seeds. KI, which normally peptized a larger percentage of the total nitrogen than either of the other salts, peptizes practically the same amount of total nitrogen as KF. The iodide ion does not always occupy a paramount position in the lyotropic series. Fischer,[53] for example, using a number of salts, found the order of acid radicals bringing about a diminution in the swelling of fibrin in any acid or alkali to be $Cl < Br < NO_3 < CNS < I <$ acetate $< SO_4 <$ phosphate $<$ tartrate $<$ citrate. The sulfate ion in this case had a greater effect than did the iodide ion.

The peptization range of the *Cruciferae* is comparatively narrow, mustard ranging from 57.56 to 60.05% with a difference of only 2.49%; radish ranging from 72.36 to 77.43% with a difference of only 5.07%, and rape ranging from 67.71 to 74.61% with a difference of only 6.90%. The small differences in peptization behavior sharply distinguish the proteins of the *Cruciferae* from those of the other seeds thus far considered.

The curves for radish and rape are seen to be rather sharply differentiated from that of mustard, a much higher percentage of the total protein of the former seeds being peptized. The two curves are similar in nature which seems to indicate that the proteins of radish and rape have responded in an analogous manner to the action of the various salt solutions.

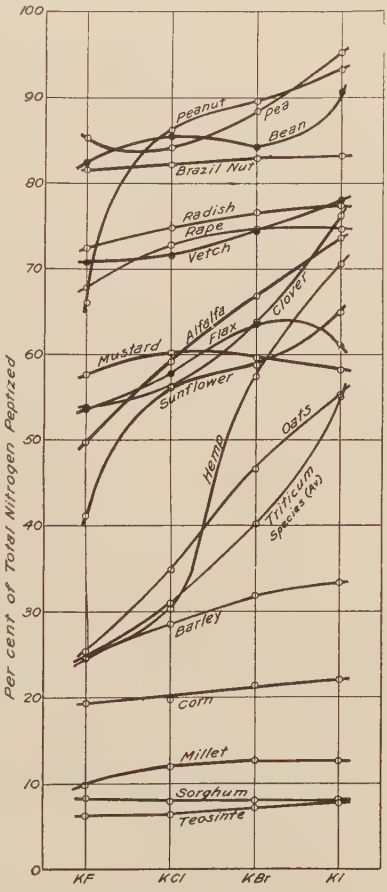

FIG. 2

A Composite Graph showing the Peptization of the Proteins of Various Seeds and Grains by 0.5 M. Salt Solutions

Peptization of the Proteins of Miscellaneous Seeds.—The seeds appearing under this title have been classified into two groups. The first four, yellow dent corn, white Ural millet, sweet sorghum, and teosinte, are in reality cereals and fall rather naturally into a group by themselves. Brazil nut, flax, hemp, and Russian sunflower make up the second group. The first four seeds will be designated collectively for the purpose of the present discussion as the "corn group."

(a) *The Corn Group.*—In many ways the "corn group" of seeds is the most interesting that has thus far been discussed. The entire group is characterized by having a very low percentage of peptizable protein. None of the

other seeds or grains which have been studied in this laboratory even approach these low values. It is also noteworthy that the variation between the effect of the different salt solutions when a single seed variety is considered is in most cases of very small magnitude. It is obvious that in this group we are dealing with proteins having properties of an entirely different nature than was found, for example, in the case of the proteins of the other cereal grains.

If we list the anions in order of their lyotropic effect, the following series are found for the different members of the corn group: yellow dent corn and teosinte, $F < Cl < SO_4 < Br < I$; white Ural millet, $F < SO_4 < Cl < Br < I$; sweet sorghum, $SO_4 < Cl < Br < I < F$. *Actually*, however, these orders do not give the true picture, since the values for a number of the salts, considering a given seed variety, lie within experimental error of one another. Thus the percentages of the total protein of corn peptized are practically the same regardless of whether KF, K_2SO_4, or KCl solutions are employed. The lyotropic order accordingly would be more correctly indicated as F, SO_4, $Cl < Br < I$. In the case of millet, the salts, K_2SO_4 and KCl, have equal peptizing power, as have also KBr and KI; therefore, for this seed $F < SO_4$, $Cl < Br, I$. When the data for sorghum seed are considered, it is apparent that no lyotropic series exists. The same value is obtained in all cases, regardless of whether KF, K_2SO_4, KCl, KBr, or KI are used as the peptizing agent.

KF, K_2SO_4, and KCl peptize about the same percentage of the total nitrogen of teosinte, while KBr and KI peptize larger amounts; therefore, F, Cl, $SO_4 < Br < I$.

A graphical representation of the data for corn, millet, sorghum, and teosinte is included in Fig. 2. With the exception of millet, straight lines have been drawn connecting the points. The line for corn is separated from the other lines or curves, inasmuch as a higher percentage of its total nitrogen was peptized than was true of other members of the group. While teosinte and corn differ in the relative amounts of nitrogen peptized, yet it will be observed that the lines slope in the same direction. However, the angles which these lines make with the horizontal are not the same, the angle being greater in the case of corn.

(b) *The Proteins of Other Seeds.*—The peptization data for the second group of miscellaneous meals are also found in Table I and illustrated graphically in Fig. 2. The lyotropic series found for each of these seeds are Brazil nut and hemp, $F < Cl < SO_4 < Br < I$; flax, $F < Cl < I < Br < SO_4$; and sunflower, $F < SO_4 < Cl < Br < I$.

The percentage of the total protein peptized ranges from 81.58 to 83.19 with a difference of only 1.61% in the case of the Brazil nut; from 53.53 to 65.26 with a difference of 11.73% in the case of flax; from 41.18 to 64.90 with a difference of 23.72% in the case of sunflower, to 24.65 to 70.58 with a difference of 45.93% in the hemp seed meal.

The proteins of the Brazil nut are very easily peptized and resemble in this respect the proteins of the pea. With the exception of the position of the sulfate ion, the lyotropic series resembles that found for the cereal grains.

The peptization range, however, is much smaller, a difference of only 1.82% being found between KF and KI. Fig. 2 shows that a straight line can be drawn connecting all the points.

A very peculiar arrangement of the ions is found in the case of the flax-seed meal. While the fluoride solution peptizes the smallest quantity of protein, the sulfate instead of the iodide ion peptizes the largest amount. Perhaps the presence of gums or mucilages (typical lyophilic colloids) in the flax seed mask in some way the real lyotropic effect on the protein complex.

The data for hemp seed show that the sulfate solution peptizes approximately 16% more protein than does the chloride solution. However, the halogens follow the normal order. Hemp seed is especially interesting because of its great peptization range. KF only peptizes 24.65% of the total nitrogen, while KI extracts 70.56%. This difference of nearly 46% represents the greatest extreme of any of the seeds studied.

The sunflower seed has exactly the same lyotropic order of ions as was found for the cereals. The range in the percentage of total nitrogen peptized is also of the same order of magnitude as was found for oats and wheat.

The Albumin and "Globulin" Fractions of the Cereals.—Under the conditions of the experiment, water peptizes 15.12% of the total nitrogen of barley. Of this amount 3.74% is soluble in water and probably represents to a large extent the albumin fraction. The remainder, 11.38% may represent "globulins." Column (C) of Table II shows that following three water extractions, three extractions of the residue with 0.5 M KCl will dissolve an additional 7.05% of the total nitrogen. This added to the "globulin" presumably peptized by virtue of the salts originally present in the seed, gives a total of 18.43%. Thus, "globulins" amounting to 18.43% of the total nitrogen content were peptized by the combined effect of water and salt. To this we must add 3.74% to obtain the total amount of "globulin" and albumin peptized. The figures appearing in Column (G), Table II, were taken from the peptization experiments and show the percentage of total nitrogen peptized, using a KCl solution alone (no previous water extraction). Providing the protein was not denatured by water during extraction, the results given in Column (F) should be expected to check closely with those of Column (G). Column (H), which represents the difference between Columns (F) and (G) is a measure of the extent to which the protein was denatured by water previous to peptizing the meal residue with KCl. It is seen that 6.33% of the total protein of barley was denatured by water and was thus not peptizable by subsequent treatment with KCl solution.

While water extracts from oats but little more of the total nitrogen than it does from barley, nevertheless only 1.73% of the total nitrogen is water soluble as compared to 3.74% in barley. The apparent percentage of "globulin" is thereby increased. Two separate sets of figures appear for oats in Columns (C), (E), (F), (G), and (H). The top number in Column (C) shows the percentage of the total nitrogen peptized by an M/2 solution of KCl from the residue remaining after three extractions had previously been made with water. The lower figure indicates the percentage of the total nitrogen

extracted by an M/2 solution of KBr following three water extractions. KBr, as would be expected, extracts a larger percentage of the total nitrogen than does KCl.

Osborne[9] found the proteins of oats to be rather easily denatured by dialysis. This conclusion is substantiated by Column (H), Table II. It is seen that 11.38% of the total nitrogen of oats was rendered non-peptizable by KCl and 20.59% by KBr, following a previous water extraction.

The data recorded in Table II for wheat (average) represent the average of all the results obtained from the various varieties and types of wheat, and were taken from Table V. It is included in Table II for purposes of comparison. The water-soluble fraction following dialysis is larger than for either oats or barley, although the per cent of "globulin" peptized is about the same as that for oats. Column (H) shows that the proteins of wheat are not so easily denatured by water as are those of barley or oats.

Table V contains the albumin-"globulin" data from a study made of a number of different types and varieties of wheats. The percentage of the total nitrogen peptized by water varies from 16.67% to 24.94%. The common wheats, Marquis and Quality, have 21.21 and 24.94%, respectively, of the total nitrogen which is peptized by water. Considerable variability is seen in the percentage of total water-soluble nitrogen. This ranges from Einkorn with 2.77% to Marquis with 7.04%. The "globulin" fraction remains fairly constant at 13 to 15% of the total nitrogen except for Spelt and Quality where values of 19.23% and 21.16% were found

The rather unusual behavior of Quality toward salt solutions is indicated in Fig. 1. The data of Table V show that it likewise ranks much higher in "albumin" and "globulin" nitrogen than any of the other cereals. This fact alone may account for the unique position occupied by this cereal in Table III and Fig. 1. However, in addition to Quality containing more albumin and "globulin," it is probable that its other proteins are more easily peptized by the salt solutions than is the case for the other wheats.

Column (H) of Table V is calculated in the same manner as the corresponding column in Table II and may be considered as an index of the degree to which the proteins of these cereals are denatured by water. It will be observed that the proteins of Alaska, Einkorn, and Emmer, are not easily denatured. Those of Little Club, White Polish, and Spelt are intermediate. The proteins of Mindum and the *T. vulgare* group, however, are more easily denatured, an average difference of about 3% being found between Columns (F) and (G).

The Albumin and "Globulin" Fractions of the Legumes.—The legumes (Table II) vary from 25.16 to 78.87% in the percentage of the total nitrogen extracted by water, the former figure representing Alsike clover and the latter Andes pea.

It is the opinion of most investigators that legume seeds contain large amounts of stored albumin. However, the term "albumin" seems to have had different interpretations placed upon it and has been rather loosely used. The generally accepted definition of an albumin is that of "a simple protein

soluble in water and dilute salt solutions and coagulable by heat." Mann[54] however states that, "most of the vegetable albumins are globulins, *i.e.*, acid-albumins which are insoluble in pure water, but soluble in salt-solutions, and precipitable by dilution and acidification."

If we accept the present general opinion that albumins are proteins soluble in pure water, then our results as given in Column (B), Table II, do not substantiate the belief that legume seeds contain large amounts of albumin. It will be seen that navy beans contain only 14.11% of water-soluble nitrogen which is the largest amount found for any legume. Alfalfa seed contains only 3.29%.

Jones et al[22] isolated 1.75% of an albumin from the Lima bean. Only traces of an albumin were found by Johns and Waterman[23] in the Mung bean. Osborne and Campbell[24] found the legumin content of the pea to be 4%, lentil 10%, vetch 2.5%, and the horse bean 16% based on the weight of the meal used. On the other hand, Johns and Jones[29] found but a very small quantity of albumin in the peanut. These data indicate a considerable variation in the albumin content of legumes, just as we have shown in Table II. Osborne and Campbell found about twice as much legumin in the pea as in the vetch. Our figures show somewhat roughly the same relationship. In the case of peanut, however, we find 5.36% of the total nitrogen to be water-soluble, whereas Johns and Jones found but a very small quantity. A strict comparison, however, is hardly justified because of the difference in methods used and possibly differences in the samples employed for investigation, but it does indicate the need for further quantitative work on the proteins of the legumes.

Table II shows that the "globulin" fractions of the legumes differ considerably. The "globulins" extractable by water from Alsike clover meal amount to 21.74% of the total nitrogen, and from the pea meal to 68.01%. The total globulin content of these seeds as recorded in Column (E) gives clover 35.79% and peas 78.10%. In this respect alfalfa and clover resemble each other, whereas the bean and pea are grouped together at the other extreme.

Some very interesting relationships may be seen in Column (C). Peptization of the alfalfa meal residue with KCl solution following the water extractions peptizes only 8.52% of the total nitrogen. On the other hand, under the same conditions, 35.62% of the total nitrogen of the vetch seed is peptized. This amount is even greater than was peptized originally by water (25.48%). Water has a decided denaturing effect on the proteins of the legumes as may be seen by reference to Column (G), where a KCl solution peptized 71.48% of the total nitrogen, or over twice as much as was peptized by KCl following earlier water extractions.

That the legume "globulins" are denatured by water during dialysis is shown in Column (H). The proteins of the pea are less readily denatured than are those of the other leguminous seeds. The proteins of clover show the greatest denaturization effect.

It was found to be practically impossible to extract 6-gram samples of alfalfa and clover meals with 50 cc. of KCl solution. The presence of gums made it impossible to sediment the residual meal. Accordingly the percentages obtained by peptizing 0.5 gram portions of these meals have been inserted in the table in order that a comparative study might still be made in spite of the possibility that the difference in the size of the sample may have influenced to some extent the percentage peptized, and that the values appearing for alfalfa and clover in Columns (F) and (G) may not, for that reason, be strictly comparable with the figures for the other meals.

The Albumin and "Globulin" Fractions of the Cruciferae.—Only rape and radish seed meals were included in this phase of the problem. Water peptizes about the same percentage of the total nitrogen from both of these seed meals. They differ, however, in the percentage of the total nitrogen appearing as albumins. Column (B) shows that 8.46% of the total nitrogen of the radish seed peptized by water may be true albumin, whereas with rape the corresponding figure is 12.37%.

The Albumin and "Globulin" Fractions of the Corn Group.—These seeds form an isolated group of Table II when the various nitrogen fractions are considered. Only from 4.98 to 12.58% of the total nitrogen is extractable by water. These are the lowest values for any group of seeds studied. Of the four seeds, corn ranks highest in this respect and teosinte lowest. These seeds stand in Table II in almost the same relative order as they did when compared upon the basis of the percentage of their total nitrogen which was salt-soluble (Table I). Millet is an exception, however, and does not yield its nitrogen so readily to water as does sorghum seed.

Considerable difference between the four seeds is noticed when they are compared on the basis of the percentage of total nitrogen appearing in the albumin fraction. In this respect sorghum and teosinte have identical amounts (0.83%), with millet 1.43%, and corn 3.34%.

In percentage of total "globulin" the members of this group stand in exactly the same order as they did in Table I. It should be noted that the "globulin" content of corn is from twice to three times that of millet, sorghum, and teosinte, respectively. These seeds are also outstanding when their stability toward water is considered. The proteins of corn, sorghum, and teosinte are very stable, while the proteins of millet undergo some denaturation.

The Albumin and "Globulin" Fractions of Other Miscellaneous Seeds.—These seeds include the Brazil nut, flax, hemp, and Russian sunflower.

Brazil nut resembles vetch in that the salt extraction of the residue from the water extraction dissolved more nitrogen than was peptized by the water previous to treatment by KCl solution. Flax has 64.07% of the total nitrogen peptizable by water, of which 15.64% is albumin. Attention is called to Column (H), Table II, where a negative value of 16.81% is recorded. In the discussion of Table I we noted that the presence of gums in flax meal undoubtedly masked the lyotropic effect which might have been obtained had the gums not been present. The value of 57.64% which appear in Column (G)

is, for that reason, probably too low. The figure given in Column (H), therefore, probably reflects in some manner the effect of the presence of gums upon the peptization behavior of the flax proteins.

The Effect of Different Reagents on the Total Nitrogen peptized, using the Same Charge of Sample.—Following the water and KCl peptization of a number of the samples listed in Table II, it was decided to re-extract the residues in the same manner, using 0.5 M KBr solution. No attempt was made to remove the small amount of KCl held by the moist residue before adding the KBr solution. The data are recorded in Table VI.

It will be seen that following the extractions by water and KCl, the addition of a 0.5 M KBr solution to the residue of barley peptized an additional 3.38% of the total nitrogen. The total amount extracted by water, KCl, and KBr was 25.55% of the total nitrogen. Column 6 shows that the water peptized 2.14 times as much of the total nitrogen of barley meal as did KCl following the water extraction. Column 7 indicates that KCl extracted 2.09 times as much nitrogen as did the subsequent extraction with KBr. Similarly 4.47 times as much nitrogen was extracted by water as was peptized by KBr following extraction by KCl.

An interesting relationship will be noted in the case of oats. Two samples of this meal were first extracted with water, 16.77% of the total nitrogen being peptized in the one case and 16.81% in the other. From this point on, the procedure was varied. Sample (1) was further extracted with KCl, followed by KBr. Sample (2) was further extracted with KBr followed by KCl. Column 5 shows that it makes little difference which salt directly follows the water extraction, since the total nitrogen extracted by water, KCl, and KBr totals about the same in either instance.

"Globulin"/Albumin Ratios from the Water Extracts.—The last columns of Tables II and V show the "globulin"/albumin ratios as obtained from all of the seeds studied. It will be observed that no two of the ratios are exactly alike. Even the cereal grains, which might have been expected to have had similar ratios, vary widely.

"Globulin"/Albumin Ratios from the Salt Extracts.—We have already suggested that the particular method employed in estimating the albumin and "globulin" fractions seemed to determine very largely the ratios found by other workers between these two proteins. In order to test this point further it was decided to make a number of salt extractions in exactly the same manner as was employed in the instance of the water extracts. The samples chosen for the study were corn, millet, sorghum, teosinte, and Brazil nuts. The results are shown in Table VII. Column 5 gives the ratios as computed from the salt extracts and Column 6 contains the ratios found from the water extracts. The disagreement between the two sets of values is most striking. Since exactly the same procedure was employed in both cases except that a different solvent was used, one must conclude that these differences are the result of employing two different methods. We feel justified, therefore, in concluding that our results substantiate what has been found by others, although never sufficiently emphasized, *i.e.* that the technic which is em-

ployed will determine to a large extent the ratio which one may expect to find between the globulin and albumin fractions of a seed. As to which method should be used of the many that might be considered, no data are at present available.

Does Elimination of the Ether-Extractable Material from a Seed Increase the Percentage of Peptizable Nitrogen?—This study included four legumes, beans, peas, alfalfa, and clover, together with hemp and corn. The percentage of ether extract ranged from 0.61% for the hemp meal which had previously been partially extracted with gasoline, to 11.72%. The data are shown in Table X.

It will be noted that with the exception of the alfalfa meal a greater percentage of the total nitrogen was peptized from the fat-free meal than from the whole seed meal. To explain the differences noted, one has only to assume that a part of the proteins of the seed are imbedded in or surrounded by a layer of fat or fat-like substances. Such an imbedding would act to prevent the ions of the salt from coming in contact with the proteins and thus exerting their maximum peptizing effect.

Protein Classification and the Lyotropic Series.—Gortner, Hoffman, and Sinclair[45] were the first to object to a system of protein classification based upon "solubility." Regarding the globulin fraction, they definitely raised the question of what salts should be used and what concentrations. As a result of their studies on twelve different wheat flours and solutions of twenty-two different inorganic salts, they state:* "We do not believe that any salt used, in any one of the concentrations, extracts a chemical entity which should be designated by the term 'globulin.' Our experiments would indicate that all of the various protein fractions (with the exception of the proteose) which have been considered fixed entities, and characteristic of wheat flours are, if we follow the prescribed routine procedure for protein isolation, capable of realization, but that, if the routine procedure is somewhat altered, fractions having different properties and characteristics are obtained." The recent work of Pascoe, Gortner, and Sherwood,[46] and Sinclair,[49] as well as the data reported in this paper have added confirmatory evidence to the findings of Gortner, Hoffman, and Sinclair.

Perhaps certain seeds or plant tissues may contain true chemical entities which can be designated as globulins. The peptization behavior of our Brazil nut meal indicates that a true globulin may be present in that seed and there are probabilities that the same may be true for radish, rape, and mustard seeds. It may be that only "crystalline" globulins are chemical entities and that the amorphous globulin preparations recorded in the literature are non-homogeneous fractions whose chemical and physical properties depend upon the technic employed in their isolation. However, the peptization behavior of the hemp seed meal certainly raises the question as to which salt solution isolates the chemical entity, "crystalline edestin;" which question can only be answered by further investigations.

* It should perhaps be emphasized that their statement has particular reference to the proteins of the wheat endosperm.

Many years ago Ritthausen questioned the homogeneity of the albumin fraction as prepared from vegetable seeds. He suggested that the substance coagulated by boiling the aqueous extract of seed meals might belong to other groups of proteins which had to some extent been "dissolved" in the water. The protein literature to date renders questionable the existence in seeds of a true albumin with constant physical and chemical properties, such as characterize egg albumin. Therefore the preceding discussion of the varying albumin:globulin ratios with varying technic applies with equal force either to the "albumin" or the "globulin" fraction.

Only recently Blish and Sandstedt[55] have questioned the chemical individuality of the protein, glutenin, of wheat. They note that boiling 70% alcohol peptized glutenin and that six successive extractions of 20 grams of supposedly "pure" glutenin, each with one liter of boiling 70% alcohol peptized a total of 25.9% of the glutenin. Even after these six successive extractions more of the residue could be peptized by further extraction. However, the most striking feature of their work lies in the fact that *the composition (as expressed by a Van Slyke analysis) of the protein peptized varied from extract to extract* and also from the Van Slyke analysis of the original glutenin. They conclude: "It appears more than likely that 'glutenin,' as ordinarily prepared by the use of dilute alkali, is not a naturally occurring individual protein, but merely a 'fraction' derived through the action of the alkali, and varying somewhat in composition according to the strength of the alkali used, as well as with the time and temperature of extraction."

Glutenin has been regarded by workers in the field of the vegetable proteins as one of the most completely studied and characterized of the plant proteins. Osborne undoubtedly regarded it as a chemical entity so that in the light of the observations of Blish and Sandstedt, Osborne's[48] (p. 13) statement, "We can, therefore, for the present, treat as individual proteins only those products whose extensive fractionation has given no evidence that a mixture is being dealt with, and we must await new methods of study before any one of these proteins can be definitely accepted as a true chemical entity. Many, however, of the proteins with which we are at present familiar have shown such constancy of composition and properties that we feel justified in now considering them as substances of reasonably definite character," becomes doubly significant.

The above discussion has been directed primarily at the problems facing the worker in the field of the vegetable proteins. Whether or not they apply with equal force to the "proteins" isolated from animal fluids, cells, and tissues can only be answered by studies in which the animal fluids or tissues are peptized under a variety of conditions. However, the recent observations of Lustig[56] appear to be significant. Using non-standard technic Lustig isolates from blood serum three euglobulins, two pseudoglobulins, and three albumins! These preparations differed sharply from each other in both physical and chemical properties. If Lustig's fractions represent chemical entities, then the earlier preparations of the serum proteins were non-homogeneous. But there is no evidence that Lustig's preparations cannot be further fractionated

by other technics. The whole question of protein individuality, protein classification, and protein isolation needs to be critically studied, and the workers in this field should divorce themselves from all preconceived notions as to what constitutes orthodox or non-orthodox technics.

Abderhalden[57] has drawn attention to the fact that the proteins as we work with them in the laboratory are not in the same physical state as they were originally in the cell. In fact the significant role they are called upon to play in vital phenomena depends upon their easy convertibility and sensitivity. He states that certain of the proteins in protoplasm undoubtedly have properties that are still unknown to us.

If Abderhalden is correct, then a number of the nitrogen-containing preparations which we term proteins may not in reality be distinct entities, but may be simply fractions of a large structure of which we know very little. From this viewpoint chemical reagents may, in many instances, merely bring about the peptization of a larger micelle into certain of its component parts, the properties and composition of which are determined by the reagent and technic which were used. Thus in the peptization of proteins by water, salt solutions, dilute alcohol, dilute alkali, etc., it may be that we are only fractionating a single primary substance and by changing to other reagents (*e.g.*, other salt solutions) another series of different fractions of this same primary substance may be obtained.

Summary

A study has been made of the peptizing effect of M/2 aqueous solutions of certain inorganic salts on the proteins of twenty-eight different seeds and grains. The investigation included barley, oats, and wheat, six different legumes, three *Cruciferae*, and other miscellaneous seeds. At least one variety of each of the eight principal types of wheats was investigated.

The data as presented seem to warrant the following conclusions:

1. A definite lyotropic or Hofmeister series of anions has been observed as influencing the peptization behavior of most of the seed protein complexes.

2. Barley, oats, and all of the varieties and types of wheats studied have the same lyotropic series, the anions arranging themselves in the order of increasing peptization effect of $F < SO_4 < Cl < Br < I$. Some variation is found in the case of the legume seeds. If the halogens alone are arranged, the order is essentially $F < Cl < Br < I$. The lyotropic order for the pea, however, is $SO_4 < Cl < F < Br < I$. Of the *Cruciferae*, radish and rape meals have the lyotropic order of anions of $F < SO_4 < Cl < Br < I$; mustard meal, $F < I < SO_4 < Br < Cl$.

3. The lyotropic effect in the case of corn, millet, sorghum, and teosinte meals is much less pronounced than that found for the other seeds. In fact no lyotropic order of anions could be demonstrated for sorghum seed.

4. Brazil nut meal and hemp meal had a lyotropic order of anions of $F < Cl < SO_4 < Br < I$; flax meal, $F < Cl < I < Br < SO_4$; and sunflower meal, $F < SO_4 < Cl < Br < I$.

5. The peptization behavior of the various groups of related seeds appears to be rather distinct and characteristic of each group.

6. Although the same methods were employed in each case, different globulin: albumin ratios were found where extractions were made with distilled water followed by 0.5 M KCl and those where only 0.5 M KCl was used.

7. A greater percentage of the total nitrogen was extracted from fat-free meals than from the whole grain meals.

In conclusion it is emphasized that protein "solubility" is in reality protein peptization, and that the technic which is employed may largely determine the yield and the properties of the protein complex which is isolated. The existence of true plant "albumins" as definitely characterized chemical entities is questioned. The available evidence indicates that many of the preparations which have hitherto been regarded as homogeneous and distinct plant proteins may in reality either be heterogeneous mixtures or else merely fragments of a larger and more complex protein micelle.

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